

Association analysis of serotonin receptor 1B (HTR1B) and brain-derived neurotrophic factor gene polymorphisms in borderline personality disorder

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Abstract The purpose of this study was to test for association between Borderline personality disorder (BPD) and variants of the HTR1B and the brain-derived neurotrophic factor (BDNF) gene. We genotyped four HTR1B and the functional BDNF G196A marker in 161 Caucasian BPD patients and 156 healthy controls. There were no significant differences between groups in genotype or haplotype distribution of HTR1B markers or in genotype distribution of the BDNF marker. Logistic regression analyses revealed an over-representation of the BDNF 196A allele in HTR1B A-161 allele carrying BPD patients.

Keywords Borderline personality disorder · Genetics · HTR1B · BDNF · Gene–gene interaction

Introduction

Borderline personality disorder (BPD) is a complex psychiatric disorder characterized by repeated impulsive, auto-aggressive and suicidal behavior. It is widely accepted that BPD and its predominant clinical features are moderately to highly heritable (Lieb et al. 2004). However, the knowledge

about specific genetic susceptibility factors of BPD is still very limited. The serotonin 1B (5-HT_{1B}) receptor was repeatedly found to be involved in the regulation of impulsive-aggressive behavior in both animal (Sanders et al. 2002) and human studies (Sari 2004). Single nucleotide polymorphisms (SNPs) of the HTR1B gene have been associated with various impulsivity related clinical features such as suicidal behavior, substance abuse, and attention deficit hyperactivity disorder. The regulation and growth of 5-HT neurons and 5-HT signaling is strongly regulated by brain-derived neurotrophic factor (BDNF) (Martinowich and Lu 2008). The BDNF gene contains a functional G196A SNP, which results in a valine to methionine change at position 66, that leads to a reduced BDNF secretion (Chen et al. 2004). Neuroimaging studies in healthy subjects showed that the BDNF 196A allele was associated with a relative volume reduction in the hippocampus (Pezawas et al. 2004), a region that is probably dysfunctional in BPD. In the present study, we examined possible associations between BPD and SNPs of the HTR1B gene and the surrounding regions as well as also estimated haplotypes of HTR1B gene variants, the BDNF G196A SNP, and the possible interaction between HTR1B and BDNF gene variants.

Materials and methods

Patients and controls

All participants gave their written informed consent to participate in the study after a complete and extensive description. The study was approved by the local ethical committee of the Landesärztekammer Rheinland-Pfalz and is compliant with the Code of Ethics of the World Medical Association (Declaration of Helsinki). A total of 161

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patients with DSM-IV BPD (49 males and 112 females, mean age $\pm$ SD = 33.2 ± 9.4 years) and 156 healthy volunteers (46 males and 110 females, mean age $\pm$ SD = 33.6 ± 9.6 years) were recruited for this study. The Structured Clinical Interview for DSM-IV axis II disorders (SCID-II) was applied by trained research assistants to each patient. DSM-IV axis I disorders were evaluated with the Munich-Composite International Diagnostic Interview (M-CIDI). Patients were not included if at least one of the following criteria was present: (a) dementia or any other organic brain syndrome with obvious cognitive impairment, (b) doubts in the ability to give informed consent, (c) a current diagnosis of a psychotic disorder, (d) involvement in a legal procedure at the time of study participation, (e) not of Caucasian descent.

Genotyping

DNA was obtained from venous blood using standard techniques. We genotyped four HTR1B SNPs (rs11568817, rs130058, rs6296, rs6297) and the functional BDNF G196A SNP (rs6265). SNPs were genotyped using a Sequenom (San Diego, CA) chip-based MALDI-TOF mass spectrometry platform. In brief, polymerase chain reaction (PCR) and extension reactions have been designed using MassARRAY design software, and were carried out using 2.5 ng of template DNA. Unincorporated nucleotides in the PCR product were deactivated using shrimp alkaline phosphatase. The primer extension products were then cleaned and spotted onto a SpectroChip with a massARRAY nanodispenser. The chips were scanned using a mass spectrometry workstation (MassARRAY compact analyzer, Sequenom) and the resulting spectra were analyzed and genotypes were determined using the Sequenom SpectroTYPER-RT software.

Statistical analysis

In the whole study sample, for controls and cases, deviations from Hardy–Weinberg equilibrium (HWE) were tested separately by comparing the observed and expected genotype frequencies by two-tailed χ^2 -test ($df = 1$) and permutation p -values (10,000 permutations). For case–control comparison regarding HTR1B SNP genotype frequencies, the Armitage–Trend test was used. Haplotype frequencies were estimated by maximum likelihood estimation. Differences in haplotype distribution between patients and controls were assessed with the Likelihood-Ratio test (SAS Genetics) and 1,000 permutations for permutation p -value calculation. For case–control comparison regarding the BDNF G196A genotype frequencies, the Armitage–Trend test was used. To model the single and combined effects of the two gene variants, we performed different logistic regressions with the subjects' classification as patient or control as dependent

variable. First, we performed a forward stepwise modeling, offering all possible effect terms (additive, recessive and dominant) for each gene variant and all possible interactions. Then, two further logistic regressions with participants' status as dependent variable were performed (see below). For the primary hypotheses of association of the five SNPs with BPD the multiple significance levels were set at 5% and a Bonferroni correction was used to adjust for these five tests. All other p -values cannot be considered as significant and were only calculated for the purpose of description. The analyses were performed using SAS V9.1, SPSS V15 and R (version 2.6).

Results

For both cases and controls, distributions of genotype frequencies of the five analyzed SNPs did not show any divergence from HWE ($p > 0.1$ for each analysis). There were no significant differences in genotype distribution of the five markers between BPD patients and controls (all adjusted p -values > 0.05 , Table 1), and there was no overall association of HTR1B marker haplotypes and BPD (permutation p -value = 0.389).

The logistic regression model with all possible single effects and interactions of HTR1B and BDNF markers suggested an interaction between HTR1B A-161 and BDNF 196A allele carriers. To further examine this effect we calculated two different logistic regression models with the affection status (BPD patient or control) as dependent variable. In these models we examined the effect of BDNF G196A genotypes (196A and no 196A allele carriers) on BPD in HTR1B A-161 and no A-161 allele carriers. A conspicuous effect was estimated for BDNF 196A allele carriers in HTR1B A-161 allele carriers ($p = 0.039$, OR = 1.68), but not in HTR1B no A-161 allele carriers ($p = 0.517$; OR = 0.59). The genotype distributions for BDNF G196A SNP split by HTR1B A-161 and no A-161 allele carriers (see Table 2) showed an over-representation of the BDNF A196A and the G196A genotype in patients compared to healthy controls ($p = 0.0205$, Armitage–Trend test), suggesting a dominant effect of the 196A allele. This assumption was supported by the comparison of the distribution of BDNF genotype groups A196A/A196G versus G196G between patients and controls in HTR1B A-161 carriers ($p = 0.038$).

Discussion

Our analysis revealed no globally significant differences of genotype or haplotype frequencies of HTR1B markers between BPD patients and controls. The same holds true

Table 1 Genotype distribution of HTR1B and BDNF SNPs in BPD patients and controls

Locus	Genotype	Genotype frequency <i>N</i> (%)		<i>p</i> -value (adjusted <i>p</i> -value) ^a
		Controls	BPD patients	
HTR1B A1180G	GG	3 (1.9)	3 (1.9)	0.7341 (1.0000)
	GA	39 (25.0)	44 (27.3)	
	AA	114 (73.1)	114 (70.8)	
HTR1B G861C	CC	11 (7.1)	5 (3.1)	0.3010 (1.0000)
	CG	60 (38.5)	63 (39.1)	
	GG	85 (54.5)	93 (57.8)	
HTR1B A-161T	AA	71 (45.5)	78 (48.5)	0.2898 (1.0000)
	AT	68 (43.6)	73 (45.3)	
	TT	17 (10.9)	10 (6.2)	
HTR1B T-261G	GG	36 (23.1)	28 (17.4)	0.6883 (1.0000)
	GT	75 (48.1)	90 (55.9)	
	TT	45 (28.9)	43 (26.7)	
BDNF G196A	AA	3 (1.9)	8 (5.0)	0.055 (0.2775)
	AG	46 (29.5)	57 (35.4)	
	GG	107 (68.6)	96 (59.6)	

^a *p*-values are given for case–control comparison (Armitage–Trend test)

Table 2 Genotype distribution of the BDNF G196A SNP for carriers and no carriers of the HTR1B A-161 allele

	Genotype frequencies (%)		<i>p</i> -value ^{a,c}		Genotype frequencies (%)		<i>p</i> -value ^{b,c}
	Controls	BPD patients			Controls	BPD patients	
HTR1B T-161T				HTR1B T-161T			
BDNF G196G	8 (47.1)	6 (60.0)	0.5096	BDNF G196G	8 (47.1)	6 (60.0)	0.5158
BDNF A196G	8 (47.1)	4 (40.0)		BDNF A196A/A196G	9 (52.9%)	4 (40.0)	
BDNF A196A	1 (5.9)	0 (0)					
HTR1B A-161A/A-161T				HTR1B A-161A/A-161T			
BDNF G196G	99 (71.2)	90 (59.6)	0.0205	BDNF G196G	99 (71.2)	90 (59.6)	0.0380
BDNF A196G	38 (27.3)	53 (35.1)		BDNF A196A/A196G	40 (28.8)	61 (40.6)	
BDNF A196A	2 (1.4)	8 (5.3)					

^a *p*-values are given for case–control comparison (Armitage–Trend test)

^b *p*-values are given for case–control comparison (χ^2 test)

^c *p*-values are descriptive and cannot be considered significant at any level

for the BDNF G196A polymorphism. These findings do not support the hypothesis that variants of the HTR1B gene or the BDNF gene play large independent roles in the genetic susceptibility to BPD. The most important finding of this study was an association between the HTR1B A-161 and the BDNF 196A allele and BPD. BPD patients carrying the HTR1B A-161 allele displayed an over-representation of the BDNF 196A allele compared to healthy controls, resulting in an OR of 1.68 (95% CI 1.03–2.74, $p = 0.039$). This result has to be regarded as preliminary given (a) the small sample size in this subgroup, (b) that it was not adjusted for multiple testing, (c) the high risk of false positive results in any single association study. Therefore,

replications in independent and larger populations are necessary to corroborate our result. Although preliminary, the results support the hypothesis that this gene–gene interaction could play a role in the susceptibility to BPD. The functional consequences of the genetic interaction between the HTR1B A-161 allele and the BDNF 196A are not known. In vitro, the HTR1B A-161 allele has been associated with a reduced (Sun et al. 2002), but also with an increased 5-HT_{1B} expression level (Duan et al. 2003). Thus, it remains to be elucidated whether in BPD a reduction or an increase of HTR1B expression can be assumed as functional background for effects of the BDNF G196A polymorphism. The BDNF 196A allele was found

to be associated with a reduced BDNF secretion (Chen et al. 2004). Therefore, one could hypothesize a link between an impaired secretion of BDNF and an increased susceptibility for BPD. The potential involvement of BDNF in the pathophysiology of BPD gains support from the observation of the efficacy of antidepressants in the treatment of BPD because of their BDNF increasing capacity, as well as from human and animal studies implicating a decreased BDNF activity in depression and other stress-related disorders (Martinowich and Lu 2008). Decreased BDNF levels have been repeatedly reported in relation to suicidal behavior, such as in the hippocampus and prefrontal cortex of suicide victims (Dwivedi et al. 2003), in cerebrospinal fluid of patients with atopic dermatitis with suicide attempt compared to those without (Kimata 2005), and in blood plasma of suicidal depressive patients compared to non-suicidal ones (Kim et al. 2007). Furthermore, the 196A allele was found to be over-represented in Japanese major depressive patients with suicidal behavior (Iga et al. 2007). In imaging studies, the 196A allele was associated with decreased volume of hippocampus (Pezawas et al. 2004), a region probably dysfunctional in BPD patients. Taken together, our result suggests a relation between the HTR1B A-161 $\times$ BDNF 196A interaction, suicidal behavior, reduced hippocampus volume and BPD. Subject to independent replications, future studies should clarify the functional impact of the herein reported HTR1B $\times$ BDNF interaction on BPD, e.g., by neuroimaging methods and determination of BDNF levels in blood or cerebrospinal fluid. Furthermore, it should be of particular interest, whether the HTR1B $\times$ BDNF interaction modulates the risk of developing BPD symptoms as a function of life events (e.g., childhood trauma), which might lead to better understanding of the neurobiological correlates of the proposed gene $\times$ environment model in the etiology of BPD (Lieb et al. 2004).

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